COMPARATIVE LEVELS AND TYPES OF MICROBIAL CONTAMINATION DETECTED IN INDUSTRIAL CLEAN ROOMS

Services Provided in Support of the Planetary Quarantine Requirements

of the

National Aeronautics and Space Administration

Under

Contract R-137

Prepared by: Martin S. Favero

John R. Puleo

James H. Marshall

Gordon S. Oxborrow

Phoenix Field Station, Technology Branch
Communicable Disease Center
Public Health Service
U.S. Department of Health, Education, and Welfare
Phoenix, Arizona

Report No. 9

December 9, 1965

COMPARATIVE LEVELS AND TYPES OF MICROBIAL CONTAMINATION DETECTED IN INDUSTRIAL CLEAN ROOMS

ABSTRACT

The primary objective of this study was to determine quantitatively and qualitatively the predominant types of microbial contamination occurring in conventional and laminar flow clean rooms. One horizontal laminar flow and three conventional industrial clean rooms and three open factory areas were selected for microbiological tests. The results showed that as the environment and personnel of a clean room were controlled in a more positive manner with respect to the reduction of particulate contamination, the levels of airborne and surface microbial contaminants were reduced accordingly. The chief sources of microbial contamination were associated with the density and activity of clean room personnel. In addition, the vast majority of microorganisms isolated from the intramural air by air samplers were those indigenous to humans.

Studies on the fallout and accumulation of airborne microorganisms on stainless steel surfaces showed that although there were no significant differences in the levels of microbial contamination among the conventional clean rooms, the type of microorganism detected on stainless steel surfaces was consistently and significantly different. In addition, the "plateau phenomenon" occurred in all environments studied. It was concluded that the stainless steel strip method for detecting microbial accumulation on surfaces is efficient and sensitive in ultra-clean environments and is the most reliable and practical method for monitoring microbial contamination in future class 100 clean rooms to be used for the assembly of space hardware which will be sterilized.

INTRODUCTION

The National Aeronautics and Space Administration (NASA) requires that spacecraft hardware designed to impact or orbit Mars be sterile. The chances for transporting viable terrestrial organisms to Mars must be less than one in ten thousand. Since dry heat is to be employed as the means of sterilization, the probability of obtaining a sterile spacecraft is enhanced significantly if the level of microbial contamination is relatively low prior to the heat treatment. In accordance with this basic premise, it is necessary to assemble and test spacecraft, required to be sterile, in areas where the levels of microbial contamination can be maintained at an extremely low level.

The primary objective of this study was to determine quantitatively and qualitatively the predominant types of microbial contamination found in conventional industrial clean rooms and in clean rooms which employ laminar air flow to control particulate contamination. Preliminary results of this study were reported earlier (1,2). However, in order to maintain continuity, some of the earlier data are included in this report.

Experimental procedures.

Four industrial clean rooms and three general manufacturing areas involved in aerospace activities in the Phoenix area were selected for microbiological tests. Since the processes conducted in these rooms required various levels of environmental control, the provision for excluding contaminants differed in the three areas. Table 1 contains a description of the physical and operational characteristics of the four clean rooms. The three general manufacturing areas A, C, and D were open factory environments. Manufacturing area C was a machine shop. In manufacturing areas A and D

conventional electronic components were assembled, labeled, and packed for shipment. No environmental control was required for these activities.

Figures 1, 2, 3, and 4 illustrate the general layout of each clean room and indicate the sampling sites. The personnel and activities associated with clean room B were transferred to laminar flow clean room D after its construction.

The detailed methods of air and surface sampling and the technique used to enumerate the number of microorganisms which accumulate on stainless steel surfaces have been described previously (1,3,4). In each area studied, air samples were obtained during full working days with slit samplers.* Surface contamination on bench tops was measured by the Rodac plate technique (5). The accumulation rates of microorganisms on surfaces were determined by exposing sterile stainless steel strips (1" x 2") to the intramural air of the test area for a period of 21 weeks. At intervals of 3 weeks the strips were returned to the laboratory and the number of mesophilic aerobic and anaerobic microorganisms, as well as mesophilic aerobic and anaerobic spores, were determined. The culture medium used for all sampling was trypticase soy agar. Cultures were incubated at 32° C for 72 hours.

Stainless steel strips which were exposed to the intramural environment of the horizontal laminar flow clean room were assayed in a laminar flow clean bench. Also the strips were assayed at weekly rather than 3 week intervals.

Culture plates from the air, surface, and stainless steel strip samplings were selected at random and representative numbers of colonies were picked and subcultured. The cultures were gram stained and subjected to

---

* Reyniers and Sons, Chicago, Illinois.

pertinent biochemical tests and identified. In the case of microorganisms accumulating on stainless steel surfaces, 39 to 40 colonies were picked randomly after each 3-week assay period. In all, about 1,600 cultures were examined.

Results.

The air sampling results in clean rooms A, B, C, and D are presented in Tables 2, 3, 4, and 5. It is evident that in areas that did not require strict environmental control, such as clean room A, the level of viable particles per cubic foot was essentially the same as that of adjacent corridors or factory areas. However, in clean rooms B, C, and D the numbers of viable particles per cubic foot were consistently lower than in the non-controlled areas. The number of personnel and their activity influenced significantly the levels of contamination, as shown in Figures 5 and 6. Whenever personnel left the area, the levels of viable particles decreased accordingly. Figure 5 also points out that a definite level of background contamination existed even in the absence of personnel. This was usually not the case in clean rooms B (1) or C (Figure 6). Table 4 shows that in clean room C the level of viable particles increased progressively the farther the sampling site was from the laminar flow work benches. (Site A, B, and E, respectively).

The effect of shoe cleaning operations on the level of airborne contaminants is illustrated in Figure 7. Significant aerosols of viable particles were generated whenever the shoe cleaner was in operation. This particular shoe cleaner was situated in the change room of clean room C. Figures 8 and 9 show typical air sampling results obtained in the horizontal laminar flow clean room. No viable particles were detected at the filter wall. However, as the air moved past personnel toward the exhaust wall, the level of microbial contamination increased progressively. Samplers placed at floor level detected higher

levels of contamination than those placed at the six foot level. At both sampling sites, however, the number of viable particles decreased to zero when personnel left the area.

Figure 10 shows the comparative levels of viable particles per cubic foot in manufacturing areas A and C, and clean rooms A and D. The values for clean rooms B and C, which were not plotted, fall between those for clean rooms A and D.

Table 6 contains the results of the surface sampling on bench tops in several clean rooms and manufacturing areas by the Rodac plate method. In general the level of surface contamination appeared to increase after personnel had been in the area for six hours. In addition, bench tops in open factory areas contained higher levels of contamination. The types of microorganisms detected in clean rooms and on bench tops in clean rooms were mainly those indigenous to humans. Tables 7 and 8 show typical patterns. Similar results were obtained in clean rooms C and D. Few sporeformers (Bacillus spp.), molds, and actinomycetes were detected. The vast majority were Staphylococcus spp. and Micrococcus spp. which are associated with human skin, hair, and the respiratory tract.

The detailed results of tests designed to detect the levels of microbial contamination accumulating on stainless steel surfaces exposed to the ambient environments of clean rooms A, B, and C, and manufacturing areas C and D are presented in Tables 9 to 13. The comparative levels of aerobic mesophilic microorganisms are shown in Figure 11. In clean room A the level of microbial contamination increased to a maximum in the 9th to 12th weeks and then remained constant. Similar results were obtained in clean rooms B and C. In the two manufacturing areas the levels remained relatively constant throughout the 21 week exposure period.

Trays containing stainless steel strips were placed at three sites in the horizontal laminar flow clean room. One site was located at the filter wall and the other two at the exhaust wall. (Figure 4). Figure 12 shows the comparative levels of contamination at the three sites. Site B showed the highest level of microbial contamination. This was probably due to the airstream passing by more personnel who were located directly upstream. Both exhaust sites showed no significant increase in the level of microbial contamination throughout the 7-week exposure period. For the first 5 weeks of the study no viable microorganisms were recovered from stainless steel surfaces placed immediately downstream from the filter wall (site A). Between the fifth and sixth week the air handling system failed due to a loss of electrical power. Following this failure, a three-log increase in the level of microbial contamination occurred. These latter results point out the sensitivity of this sampling method and also the efficiency of laminar air flow systems.

The types of microorganisms which accumulated on stainless steel surfaces in the four clean rooms and in manufacturing areas C and D are presented in Table 14. Each value is the mean average from 7 assay periods. The pattern in each area did not change significantly throughout the entire exposure period. The vast majority of microorganisms which were found in clean room B were Staphylococcus spp., Micrococcus spp., and the Corynebacterium-Brevibacterium group. These are indigenous to human skin, hair, and respiratory tract. Few sporeformers (Bacillus spp.), molds, and actinomycetes, which are associated with soil and dust, were detected. The personnel of clean room B were most rigidly controlled with respect to apparel and occupancy (Table 1). Its environment also was the most rigidly controlled among the three conventional clean rooms.

Clean room A, which required the least amount of environmental and personnel control, showed a significantly different pattern with respect to types of microorganisms. Most of the microorganisms found to accumulate on stainless steel surfaces were sporeformers and molds. Only a small portion of the microbial population consisted of microorganisms of human origin. This same type of pattern also was present in manufacturing areas C and D

Clean room C exhibited a pattern which reflects its environmental control. The air cleaning system was good in that ultra-high efficiency filters were employed and 3 laminar flow benches were located at one end of the clean room. Personnel control, however, was similar to that in clean room A (Table 1). Although the majority of microorganisms detected were Staphylococcus spp., Micrococcus spp., and the Corynebacterium-Brevibacterium group, sporeformers as well as molds also were present in a higher ratio than in clean room B.

In horizontal laminar flow clean room D the qualitative pattern at both exhaust sites was similar to that of clean room C except that higher percentages of sporeformers were detected. These latter data point out the importance of personnel apparel. As was mentioned in the Experimental procedures, the personnel and activity in clean rooms B and D were identical. However, when the laminar flow room was used, some personnel constraints were relaxed (Table 1). For example, no air showers or plastic booties were required. These factors, especially the latter, could account for the higher number of sporeformers. In addition, tests were performed to determine the pattern of air flow in the immediate area upstream from the exhaust wall and sampling sites. Air velocities were measured with a thermo-anemometer (Alnor). The results indicated that the air pattern ceased to be laminar as it approached the exhaust wall and tended to rise faster toward the top

portion of the exhaust wall. This factor, too, could have influenced the types of microorganisms found.

Table 15 shows that very few obligate anaerobic microorganisms were detected on stainless steel strips exposed to the environments studied. The vast majority were facultative microorganisms which were able to grow both aerobically and anaerobically.

Discussion.

It is evident from the results obtained in this study that as the environment and personnel of a clean room were controlled in a more positive manner with respect to the reduction of particulate contamination, the levels of airborne and surface microbial contamination were reduced accordingly. This was especially true in the horizontal laminar flow clean room. The chief sources of microbial contamination were associated with people working in the clean room, as shown by the increase or decrease of airborne viable particles, depending on the number of personnel in the room and their activity. In addition, the vast majority of microorganisms isolated from the air by air samplers and surface top surfaces by the Rodac plate method were Staphylococcus spp., Micrococcus spp., and the Corynebacterium-Brevibacterium group. These bacteria are indigenous to human skin, hair, and the respiratory tract.

Studies on the fallout and accumulation of airborne microorganisms on stainless steel surfaces showed several interesting phenomena. Firstly, there was no significant difference in the levels of microbial contamination among the three conventional clean rooms A, B, and C. (Tables 9, 10, and 11, and Figure 11) and the exhaust wall sites of the horizontal laminar flow clean room D (Figure 12). Contamination levels were in the range of 10^3 to 10^4 microorganisms per square foot. In the two open factory areas the level of microbial contamination was

approximately one order of magnitude higher than in the clean rooms (Tables 12 and 13, and Figure 11).

Secondly, the types of microorganisms which accumulated on stainless steel surfaces were significantly different among the clean rooms and the two manufacturing areas. (Table 14). Vegetative microorganisms of human origin such as Staphylococcus spp., Micrococcus spp., and the Corynebacterium-Brevibacterium group accounted for the vast majority of microbial contamination detected on stainless steel strips in clean room B. Few microorganisms associated with soil and dust, such as sporeformers, molds, and actinomycetes were detected. In clean room A and manufacturing areas C and D the reverse situation was found. More soil types and fewer microorganisms indigenous to humans were found. Clean rooms C and D were more or less midway between these two extremes. These data definitely show that the degree of environmental control is reflected more adequately by types rather than by the number of microorganisms present. This was especially true in the conventional clean rooms. Personnel apparel, in addition to strict environmental control were the main variables which influenced these qualitative results. In clean room B the only parts of the body of personnel exposed were the eyes, nose, mouth, and cheeks. In addition, ultrahigh efficiency filters were employed and bench tops and the floor were routinely wiped down and vacuum cleaned. The personnel in clean rooms A and C wore lint-free gowns over street clothes. Clean room C, however, employed ultrahigh efficiency air filters and also contained three laminar flow clean benches.

Thirdly, the "plateau phenomenon" (1,2,3,4,6,7,8, and 9) occurred in all areas studied. The levels of microbial contamination resulting from the fallout of airborne microorganisms onto stainless steel surfaces did not increase significantly during the relatively long exposure period of 21 weeks. In some cases there was a slight increase up to 8 to 12 weeks, with levels subsequently

stabilizing. These results confirm those by other investigators in different geographical areas of the United States. One study showed that stainless steel surfaces exposed to the intramural air of an industrial clean room for one week contained the same level of microorganisms as those exposed for 52 weeks (6).

It must be emphasized that the plateau phenomenon is the result of a dynamic rather than a static system and one which is influenced by multiple factors. The most plausible explanation for the presence of a plateau is that the number of microorganisms deposited onto or surviving upon surfaces is balanced by the number of microorganisms dying on the same surfaces. It has been shown that the microbial population, especially vegetative microorganisms, is constantly decreasing on the stainless steel surfaces (2). Such factors as the absence of nutrients, relative humidity, temperature, and type of microorganism influence the survival rate of microorganisms on surfaces (10,11,12). Although mechanical or physical dislodgement of viable particles from the surfaces also may play a role, there has been no proof of it at the present time.

The results of this study also show that the use of stainless steel collecting surfaces is a much more sensitive and reliable method for assessing airborne microbial contamination in clean rooms than air samplers. This was apparent especially in the laminar flow clean room. Volumetric air samples detect airborne viable particles that have been freshly generated by personnel in the immediate area of the samplers. Since the vast majority of these are vegetative microorganisms (Tables 7 and 8) they would not survive on a surface for any appreciable length of time. The stainless steel strip method, on the other hand, assesses the fallout of airborne microorganisms onto surfaces and their subsequent survival and accumulation.

Obviously the sensitivity and reliability of the stainless steel strip method is best in an ultraclean environment. This is illustrated in Figure 12.

No microorganisms were detected until the air handling system failed. In all probability, air samplers would not have detected this failure adequately. Consequently this technique appears to be most reliable for monitoring laminar flow clean rooms where spacecraft hardware required to have an extremely low microbial load prior to heat sterilization are assembled.

REFERENCES

1. Favero, M. S., J. R. Puleo, J. H. Marshall, and G. S. Oxborrow. 1965. Detection and quantitation of microbial contamination to which spacecraft are subjected during manufacture. Report on Contract R-137, National Aeronautics and Space Administration, Washington, D. C. March 18, 1965.

2. Favero, M. S., J. R. Puleo, J. H. Marshall, and G. S. Oxborrow. 1965. Comparative levels and survival of naturally occurring microorganisms deposited on surfaces through handling and aerial fallout. Report No. 8, Contract R-137, National Aeronautics and Space Administration, Washington, D. C. August 9, 1965

3. Favero, M. S. 1965. Microbiological sampling studies at Hughes Aircraft Company. Report on Contract R-137, National Aeronautics and Space Administration, Washington, D. C. January 26, 1965.

4. McDade, J. J., A. S. Irons, and V. I. Magistrale. 1965. A microbiological survey of the Hughes Aircraft Company facilities involved in the assembly and/or testing of Surveyor spacecraft. Space Program Summary 3735, vol. 4, April 30, 1965. Jet Propulsion Laboratories, Pasadena, California.

5. Hall, L. B., and M. J. Hartnett. 1964. Measurement of bacterial contamination on surfaces in hospitals. Public Health Reports 79:1021-1024.

6. Portner, D. M., R. F. Hoffman, H. M. Decker, and C. R. Phillips. 1964. The level of microbial contamination in a clean room during a one year period. Protection Branch Report of Test No. 11-65. Fort Detrick, Frederick, Maryland.

7. Michaelsen, G. S. 1965 The bacteriology of clean rooms. Progress report on Contract NSG 643, National Aeronautics and Space Administration, Washington, D. C. March 31, 1965.

8. McDade, J. J., W. Reid, J. Christensen, D. Drummong, V. Magistrale. 1965. Microbiological studies conducted in the Experimental Assembly and Sterilization Laboratory, Space Program Summary 4:27-34. August 31, 1965. Jet Propulsion Laboratory, Pasadena, California.

9. McDade, J. J., M. S. Favero, G. S. Michaelsen. 1966. Environmental microbiology and the control of microbial contamination. In: Proceedings of the National Conference on Spacecraft Sterilization Technology. National Aeronautics and Space Administration, Washington, D. C. In press.

10. McDade, J. J., and L. B. Hall. 1963. An experimental method to measure the influence of environmental factors on the viability and the pathogenicity of Staphylococcus aureus. Amer. Jour. Hygiene 77:98-108.

11. McDade, J. J., and L. B. Hall. 1964. Survival of Staphylococcus aureus in the environment. II. Effect of elevated temperature on surface-exposed staphylococci. Amer. Jour. Hygiene 80:184-191.

12. McDade, J. J., and L. B. Hall. 1964. Survival of gram-negative bacteria in the environment. I. Effect of relative humidity on surface-exposed organisms. Amer. Jour. Hygiene 80:192-204.

TABLE 1. CLEAN ROOM DESCRIPTION

Criteria	Clean Room A	Clean Room B	Clean Room C	Clean Room D
Class at which clean room was operated	100,000[1]	II-III[2] (Closer to III)	II-III[2] (Closer to II)	Horizontal laminar flow
Size	802 ft^2	996 ft^2	930 ft^2	1,080 ft^2
Personnel apparel	Full length coats, caps	Bunny suits, hoods, booties, gloves	Full length coats	Same as Clean Room B except no booties
Personnel cleaning apparatus	None	Clothes vacuum, air showers, gloves dipped in Freon	Shoe cleaner	Clothes vacuum, gloves dipped in Freon
Personnel occupancy	12 - 22 persons 1 shift 5 days per week	6 - 8 persons, 1st shift 2 - 4 persons, 2nd shift 5 days per week	12 - 27 persons 1 shift 5 days per week	Same as Clean Room B
Air filtration	AAFG - Air Mat, 88% efficient for 5 micron size. Medium efficiency[3]	Cambridge Absolute. Ultrahigh efficiency[3]	MSA Absolute. Ultrahigh efficiency.[3] Also used laminar flow work benches	Cambridge Absolute. Ultrahigh efficiency[3]
Temperature ranges	75 ± 4° F	70 ± 5° F	72 ± 2° F	65 ± 3° F
Relative humidity	46 ± 2%	40 ± 20%	30 ± 5%	42 ± 3%
Number of air changes per hour	12 to 15	20	20	165
Function	Assembly of electronic components	Liquid oxygen valve assembly	Assembly of electronic components	Same as Clean Room B

(1) Federal Standard No. 209 "Clean Room and Work Station Requirements, Controlled Environment." 1963.
(2) U.S.Airforce Technical Order 00-25-203, "Standards and Guidelines for the Design and Operation of Clean Rooms and Clean Work Stations."
(3) Air filtration of Microbial Particles. Public Health Service Publication No. 953. 1963.

TABLE 2. AIRBORNE VIABLE PARTICLES PER CUBIC FOOT PER HOUR IN CLEAN ROOM A AND ADJACENT FACTORY AREA.

Time	First series of tests(1)				Second series of tests(2)			
	Clean room		Change room	Corridor	Clean room		Factory area	
	Site A	Site B			Site A	Site B	Site C	Site D
8 - 9 a.m.	6.36	7.40	8.96	--	2.48	3.00	5.30	14.45
9 - 10 a.m.	7.23	7.58	8.70	--	1.83	2.52	4.03	12.96
10 - 11 a.m.	5.11	6.98	7.65	--	2.33	3.50	4.41	14.15
11 - 12 a.m.	8.56	6.20	6.93	--	1.82	1.75	4.06	17.35
12 - 1 p.m.	3.10	3.91	6.86	--	1.23	1.68	3.70	13.02
1 - 2 p.m.	3.88	5.88	--	6.48	1.05	2.37	3.87	24.13
2 - 3 p.m.	2.93	4.33	--	7.00	2.38	2.95	3.87	--
Average	5.29	6.04	7.82	6.74	1.87	2.53	4.23	16.01
Range	2.93-8.56	3.91-7.58	6.86-8.96	6.48-7.00	1.05-2.48	1.68-3.50	3.70-5.30	12.96-24.13

(1) Twenty to 23 technicians present in the clean room.

(2) Twelve to 13 technicians present in the clean room.

TABLE 3. AIRBORNE VIABLE PARTICLES PER CUBIC FOOT PER HOUR IN CLEAN ROOM B AND ADJACENT CORRIDOR.

Time	Clean Room		Corridor
	Site A	Site B	
7:30 - 8:30 a.m.	1.18	1.36	11.31
8:30 - 9:30 a.m.	1.05	1.61	9.53
9:30 - 10:30 a.m.	1.20	1.38	6.98
10:30 - 11:30 a.m.	0.66	--	8.40
11:30 - 12:30 p.m.	--	1.01	7.00
12:30 - 1:30 p.m.	1.36	1.63	8.70
1:30 - 2:30 p.m.	0.60	1.18	7.03
Average	1.01	1.38	8.42
Range	0.66-1.35	1.01-1.63	6.98-11.31

TABLE 4. AIRBORNE VIABLE PARTICLES PER CUBIC FOOT PER HOUR IN CLEAN ROOM C AND ADJACENT MANUFACTURING AREA.

Time	Clean Room C			Manufacturing Area	
	Site A	Site B	Site E	Site C	Site D
8 - 9 a.m.	0.30	0.87	--	18.42	4.29
9 - 10 a.m.	0.27	0.85	--	15.99	5.20
10 - 11 a.m.	0.35	0.82	1.23	7.42	4.05
11 - 12 a.m.	0.31	1.15	2.07	11.92	3.32
12 - 1 p.m.	0.10	0.28	0.80	10.55	2.79
1 - 2 p.m.	0.20	0.85	1.02	11.42	1.93
2 - 3 p.m.	0.07	0.65	[illegible]70	9.59	5.39
Average	0.21	0.80	1.12	12.19	3.85
Range	0.07-0.35	0.28-1.16	0.70-2.07	7.42-18.42	1.93-5.39

TABLE 5. COMPARATIVE LEVELS OF AIRBORNE VIABLE PARTICLES IN HORIZONTAL LAMINAR FLOW CLEAN ROOM D.

Location of air samplers	Number of Viable Particles Per Cubic Foot*							
	Day 1		Day 2		Day 3		Day 4	
	Average	Range	Average	Range	Average	Range	Average	Range
Site 1								
6 inches downstream from filter wall	0	--	--	--	0	--	0	--
Site 2								
3 feet downstream from filter wall; 1 foot upstream from frequently used intercom	0.0023	0-0.016	0.057	0-0.017	0	--	0	--
Site 3								
32 feet downstream from filter wall	0.32	0.07-0.58	0.224	0.017-0.47	1.03	0.55-1.53	0.44	0.10-0.50
Site 4								
6 inches upstream from exhaust wall	0.64	0.22-0.93	1.15	0.58- 1.95	0.62	0.23-1.40	0.63	0.28-1.40

* Average of seven consecutive 1-hour samples.

TABLE 6. COMPARATIVE LEVELS OF SURFACE CONTAMINATION ON BENCH TOPS IN CLEAN ROOM A AND THE ADJACENT ASSEMBLY-FACTORY AREA, CLEAN ROOM B, AND CLEAN ROOM C.

Site and time of sampling	Average no. of colonies per Rodac plate	Range	No. of samples
Clean Room A			
1st test series			
Before personnel entered	12.5	2-31	20
6 hours after personnel entered	24.9	1-100	20
Clean Room A			
2nd test series			
Before personnel entered	18.7	0-63	100
6 hours after personnel entered	27.3	6-62	100
Assembly-Factory Area; site C			
Middle of work day	28.1	3-51	50
Assembly-Factory Area; site D			
Middle of work day	65.2	[illegible]-130	50
Clean Room B			
Before personnel entered	3.65	0-25	90
6 hours after personnel entered	9.89	1-32	90
Clean Room C			
Before personnel entered	9.16	0-52	100
6 hours after personnel entered	13.80	0-102	100

TABLE 7. TYPES OF AEROBIC MESOPHILIC MICROORGANISMS IN THE INTRAMURAL AIR AND ON SURFACES IN CLEAN ROOM A.

Type of microorganism	Air		Surface	
	Number	Per cent	Number	Per cent
Staphylococcus epidermidis	15	41.7	16	20.5
Staphylococcus aureus	3	8.3	12	15.4
Sarcina spp.	3	8.3	0	0
Gaffkya spp.	1	2.8	0	0
Micrococcus spp.	2	5.5	20	25.6
Bacillus spp.	0	0	6	7.7
Corynebacterium spp.	1	2.8	0	0
Flavobacterium spp.	1	2.8	12	15.4
Pseudomonas-Achromobacter spp.	5	13.9	12	15.4
Yeasts	1	2.8	0	0
Molds	3	8.3	0	0
Unidentified	1	2.8	0	0
Total no. examined	36		78	

TABLE 8. TYPES OF AEROBIC MESOPHILIC MICROORGANISMS IN THE INTRAMURAL AIR AND ON SURFACES IN CLEAN ROOM B.

Type of Microorganism	Air		Surface	
	Number	Per cent	Number	Per cent
Staphylococcus epidermidis	59	70.2	45	54.2
Staphylococcus aureus	5	5.9	0	0
Micrococcus spp.	3	3.6	10	12
Sarcina spp.	0	0	1	1.2
Gaffkya spp.	1	1.2	0	0
Streptococcus spp.	1	1.2	0	0
Bacillus spp.	0	0	3	3.6
Corynebacterium spp.	8	9.5	14	16.9
Neisseria catarrhalis	0	0	1	1.2
Pseudomonas-Achromobacter spp.	3	3.6	1	1.2
Yeasts	0	0	0	0
Molds	1	1.2	2	2.4
Actinomycetes	1	1.2	1	1.2
Unidentified	2	2.4	5	6
Total number examined	84		83	

TABLE 9. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL STRIPS EXPOSED WITHIN CLEAN ROOM A.

Weeks of exposure	Samples not heat-shocked		Samples heat-shocked at 80° C for 15 min	
	Aerobes	Anaerobes	Aerobes	Anaerobes
	No./ft^2	No./ft^2	No./ft^2	No./ft^2
3	1,728	720	936	417
6	3,168	1,[illegible]	1,944	792
9	11,664	1,296	2,520	1,656
12	6,480	3,312	2,808	2,340
15	9,072	900	3,197	418
18	12,312	3,060	2,808	1,202
21	13,968	1,138	3,060	720

TABLE 10. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL STRIPS EXPOSED WITHIN CLEAN ROOM B.

Weeks of exposure	Samples not heat-shocked		Samples heat-shocked at 80° C for 15 min	
	Aerobes No./ft^2	Anaerobes No./ft^2	Aerobes No./ft^2	Anaerobes No./ft^2
3	4,736	1,656	180	119
6	2,880	635	180	180
9	4,082	302	482	0
12	9,000	1,857	684	115
15	24,718	3,362	936	540
18	6,422	1,440	238	122
21	9,238	1,260	720	238

TABLE 11. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL STRIPS EXPOSED WITHIN CLEAN ROOM C.

Weeks of exposure	Samples not heat-shocked		Samples heat-shocked at 80° C for 15 min	
	Aerobes No./ft^2	Anaerobes No./ft^2	Aerobes No./ft^2	Anaerobes No./ft^2
3	4,918	1,318	720	122
6	8,698	2,398	1,562	648
9	7,848	1,202	1,620	238
12	16,862	1,318	2,218	720
15	26,280	1,678	3,693	302
18	18,274	1,872	5,940	1,368
21	14,818	2,398	4,248	1,678

TABLE 12. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL STRIPS EXPOSED WITHIN MANUFACTURING AREA C.

Weeks of exposure	Samples not heat-shocked		Samples heat-shocked at 80° C for 15 min	
	Aerobes No./ft^2	Anaerobes No./ft^2	Aerobes No./ft^2	Anaerobes No./ft^2
3	31,500	1,922	6,358	1,678
6	22,464	6,178	7,654	3,722
9	41,278	5,998	5,638	1,728
12	34,704	7,020	11,642	3,600
15	31,680	3,118	11,282	2,398
18	17,640	5,040	7,200	3,096

TABLE 13. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL STRIPS EXPOSED WITHIN MANUFACTURING AREA D.

Weeks of exposure	Samples not heat-shocked		Samples heat-shocked at 80° C for 15 min	
	Aerobes	Anaerobes	Aerobes	Anaerobes
	No./ft^2	No./ft^2	No./ft^2	No./ft^2
3	7,380	482	2,282	238
6	15,538	2,880	3,420	360
9	31,018	1,260	4,082	958
12	22,558	1,318	5,220	540
15	22,032	1,008	6,192	648
18	16,272	1,678	2,282	958
21	20,700	3,182	9,180	2,038

TABLE 14. TYPES OF AEROBIC MESOPHILIC MICROORGANISMS WHICH ACCUMULATED ON STAINLESS STEEL SURFACES EXPOSED TO THE INTRAMURAL AIR OF 4 CLEAN ROOMS AND 2 MANUFACTURING AREAS.

Type of microorganism	Clean Room A %	Clean Room B %	Clean Room C %	Clean Room D Site B %	Clean Room D Site C %	Manufacturing Area C %	Manufacturing Area D %
Staphylococcus aureus	0	0.5	0	0.5	0	0	0
Staphylococcus epidermidis	1.9	47.2	22.1	33.2	23.5	12.7	14.6
Micrococcus spp.	4.3	6.4	8.6	5.2	7.5	1.6	6.1
Streptococcus spp.	0	0.9	2.9	0.9	0	0	2.3
Bacillus spp. (sporeformers)	55.5	10.1	17.9	35.1	32.5	30.9	21.5
Brevibacterium-Corynebacterium group	14.3	28.4	35.7	8.5	20.5	10.3	23.8
Gram negative microorganisms	0	0	0	0.5[1]	0	15.1[1]	0
Yeasts	1.9	0.9	0.7	2.4	1.0	1.6	0.8
Molds	12.9	2.7	6.4	11.8	11.0	25.4	20.8
Actinomycetes and streptomycetes	5.7	1.4	2.1	1.9	2.0	2.4	6.9
Unidentified or lost upon subculture	3.3	1.4	0	0	0	0	0

[1] *Pseudomonas* spp.

TABLE 15. OCCURRENCE OF FACULTATIVE MICROORGANISMS AND OBLIGATELY ANAEROBIC MICROORGANISMS WHICH ACCUMULATED ON STAINLESS STEEL SURFACES EXPOSED TO THE INTRAMURAL ENVIRONMENT OF 4 CLEAN ROOMS AND 2 GENERAL FACTORY AREAS.[1]

Area	Number examined	Facultative microorganisms		Obligately anaerobic microorganisms	
		From non-heat-shocked samples	From heat-shocked samples	From non-heat-shocked samples	From heat-shocked samples
Clean Room A	32	39	13	0	0
Clean Room B	104	97	7	0	0
Clean Room C	106	76	24	6	0
Manufacturing Area C	103	62	39	1	1
Manufacturing Area D	214	167	44	3	0
TOTAL	579	441	127	10	1

[1] All cultures were isolated from plates incubated in Brewer jars at 32° C for 72 hours.

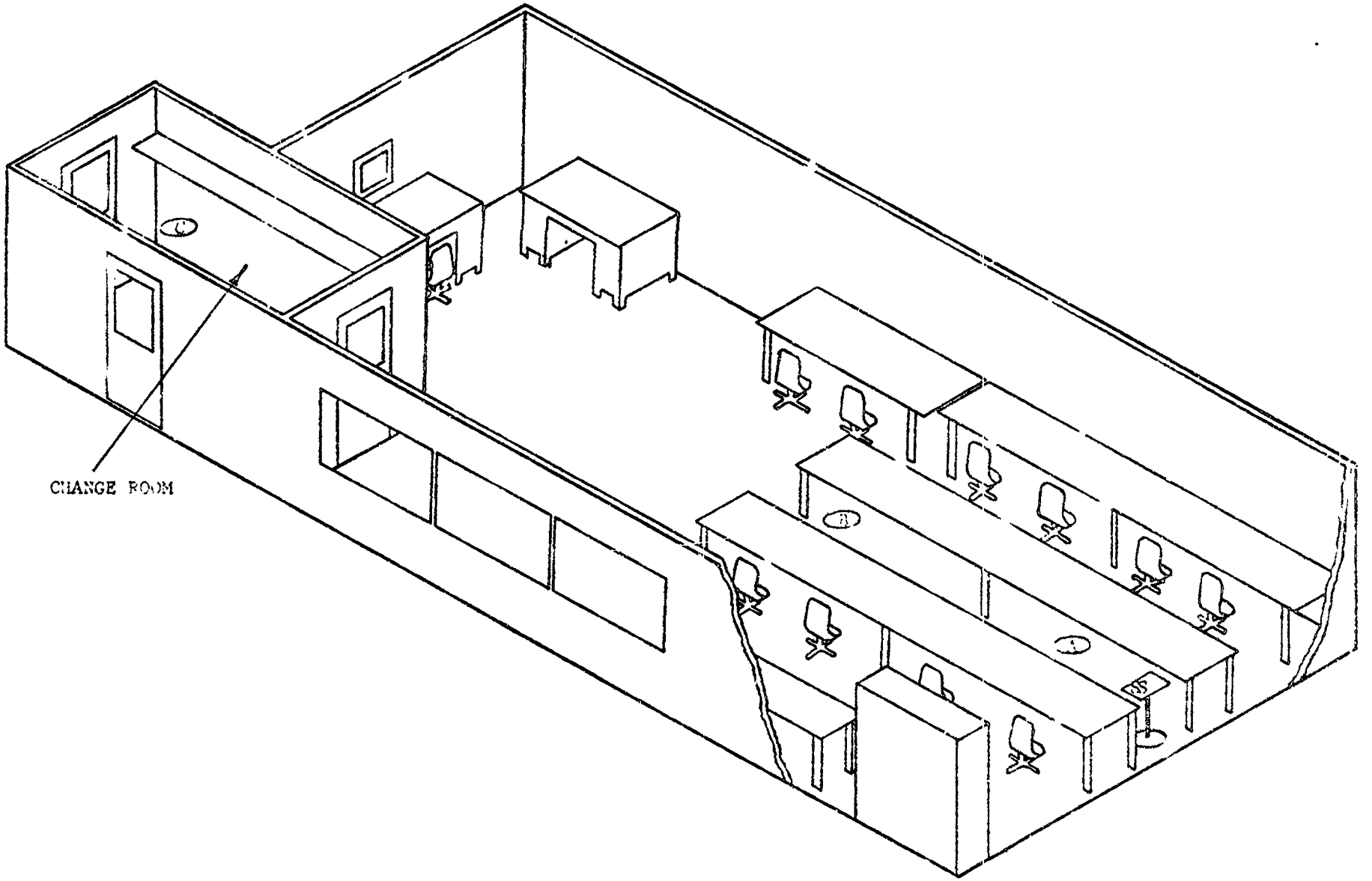


Fig. 1. General floor plan of Clean Room A. SS = Stainless steel strips; A, B, and C = air sampling sites.

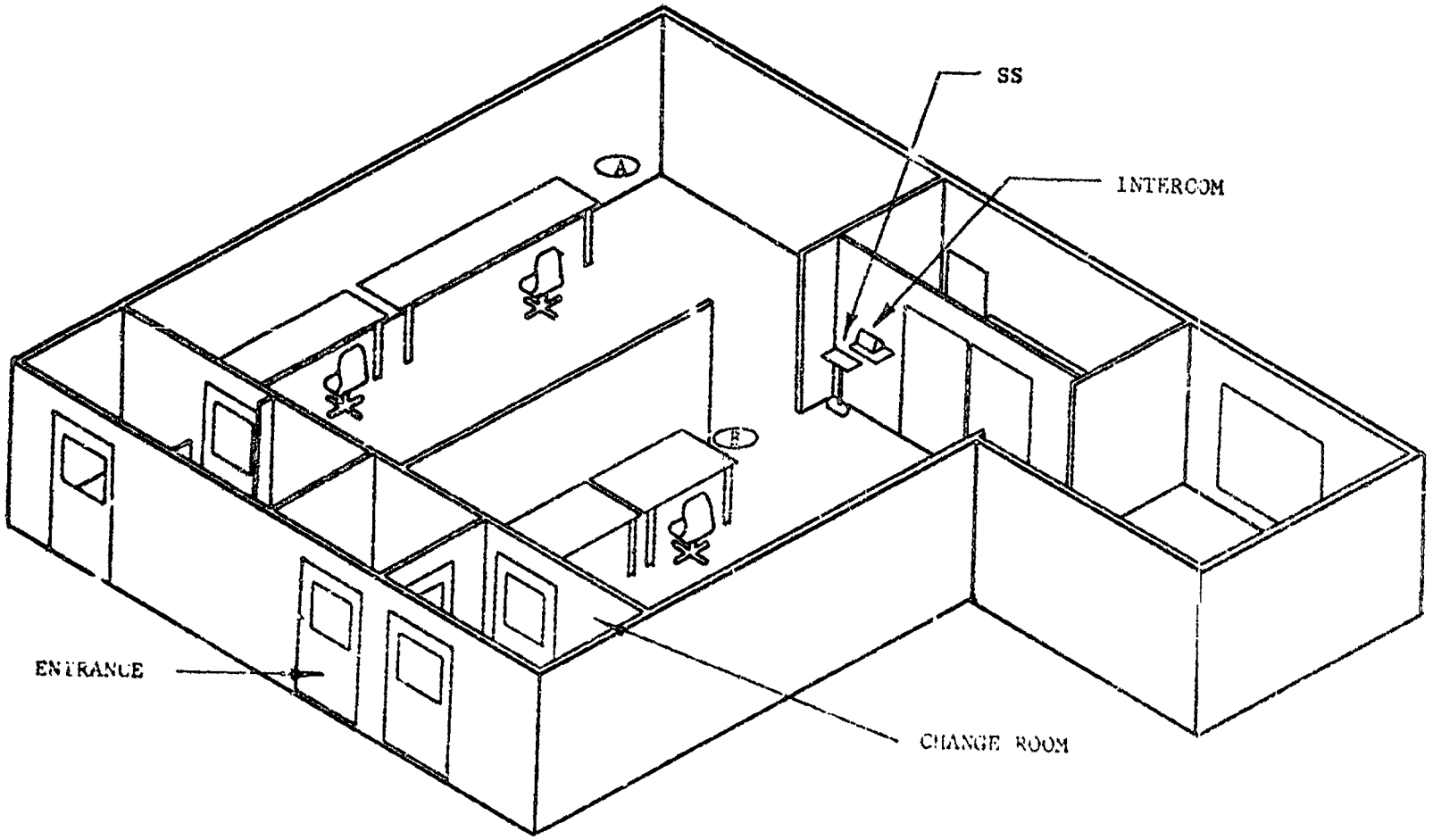


Fig. 2. General floor plan of Clean Room B. SS = Stainless steel strips; A and B = air sampling sites.

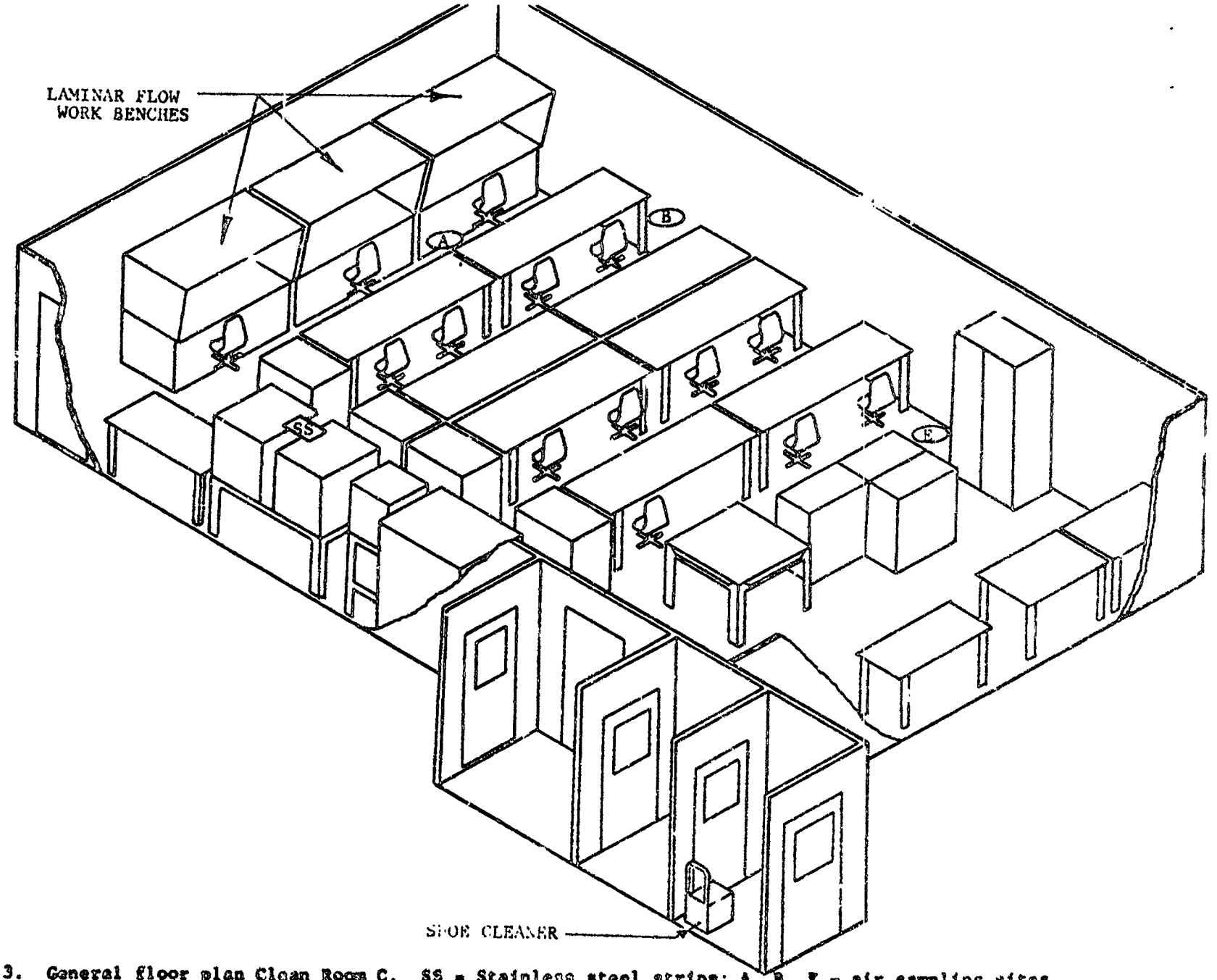


Fig. 3. General floor plan Clean Room C. SS = Stainless steel strips; A, B, E = air sampling sites.

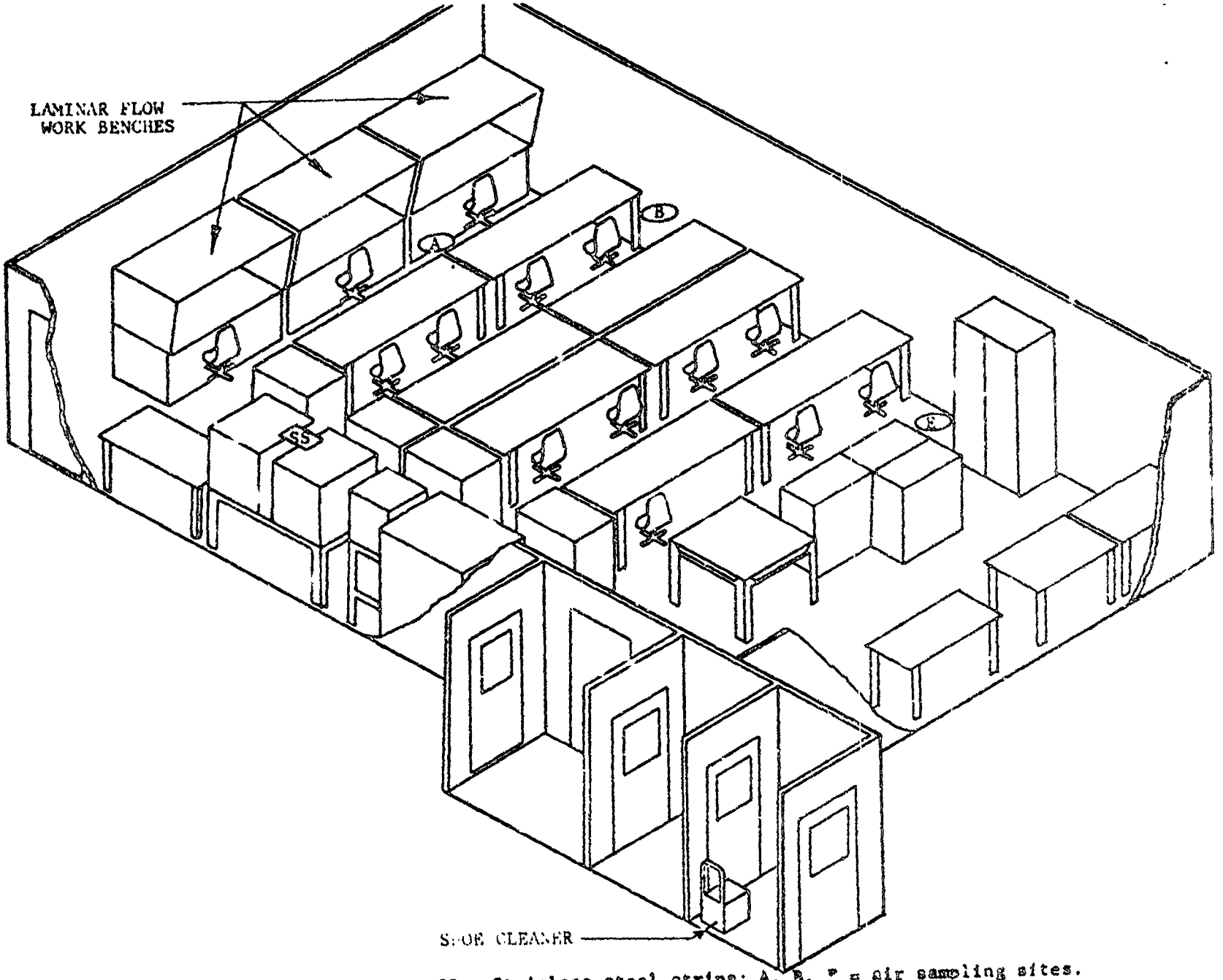


Fig. 3. General floor plan Clean Room C. SS = Stainless steel strips; A, B, ° = air sampling sites.

CLEAN ROOM D - HORIZONTAL LAMINAR FLOW

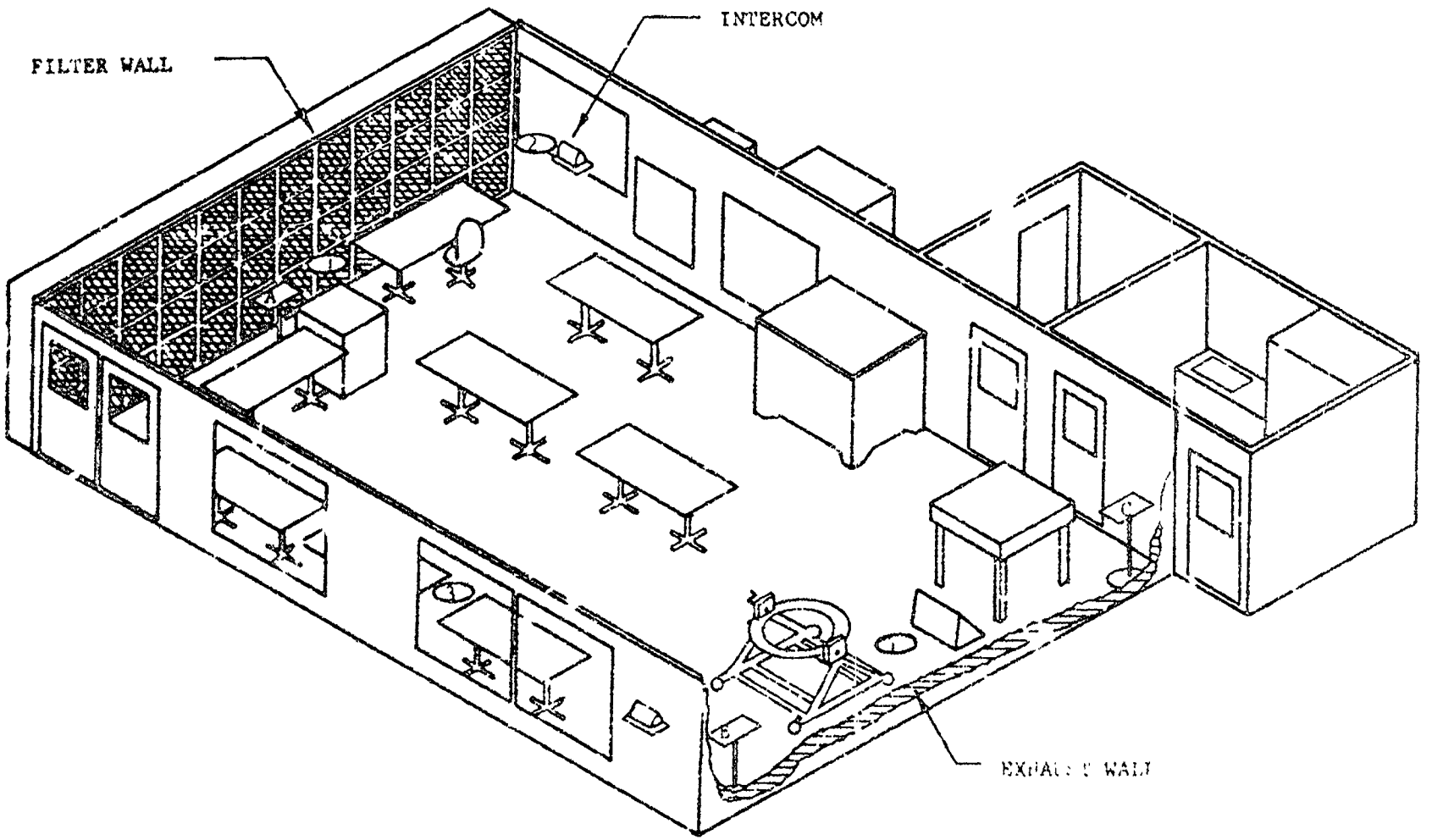


Fig. 4. General floor plan of Clean Room D. A, B, and C = stainless steel strips; 1, 2, 3, and 4 = air sampling sites.

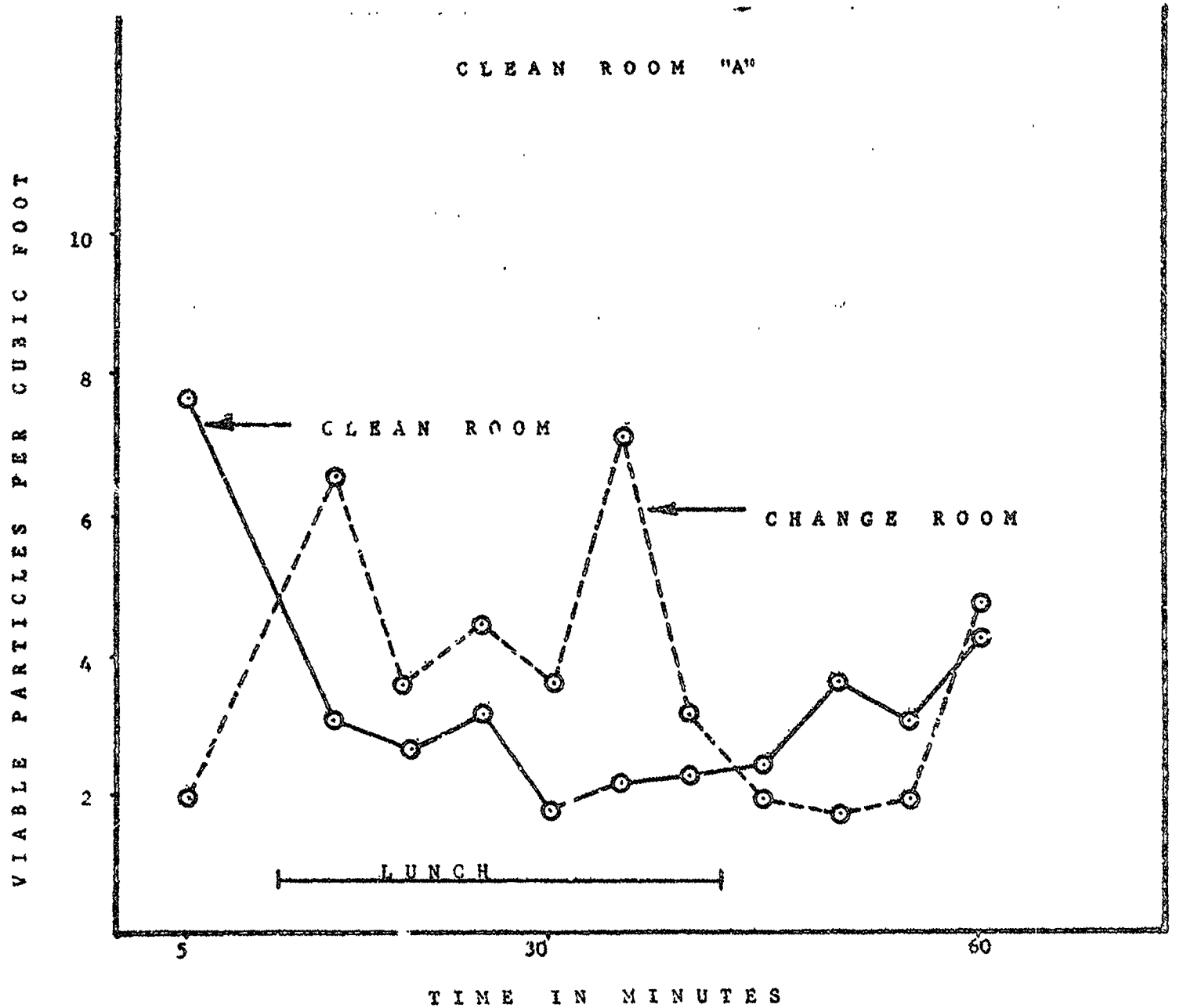


Fig. 5. Effect of personnel density and activity on the level of airborne microbial contamination in Clean Room A and adjacent change room.

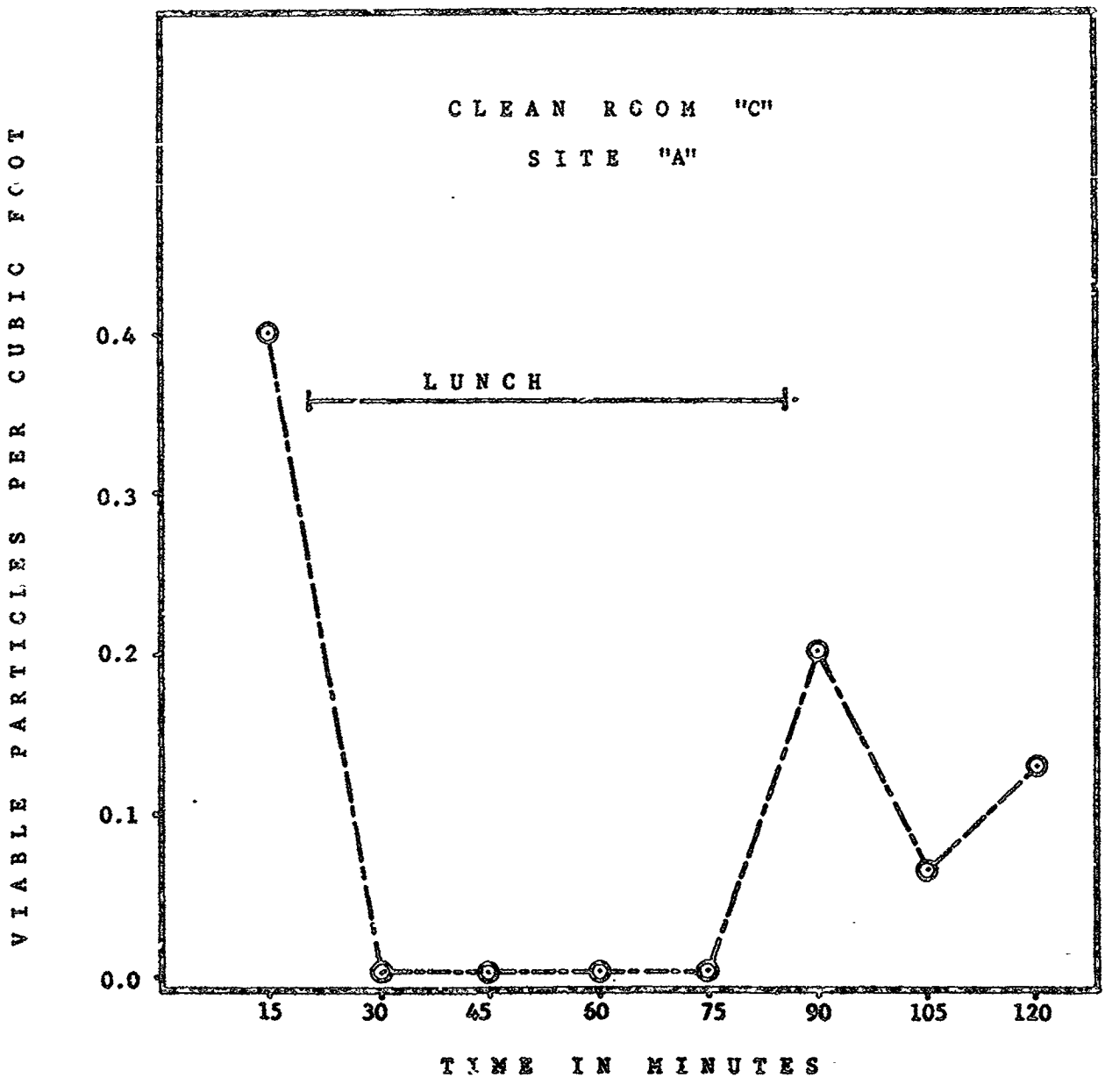


Fig. 6. Effect of personnel density and activity on the level of airborne microbial contamination in Clean Room C.

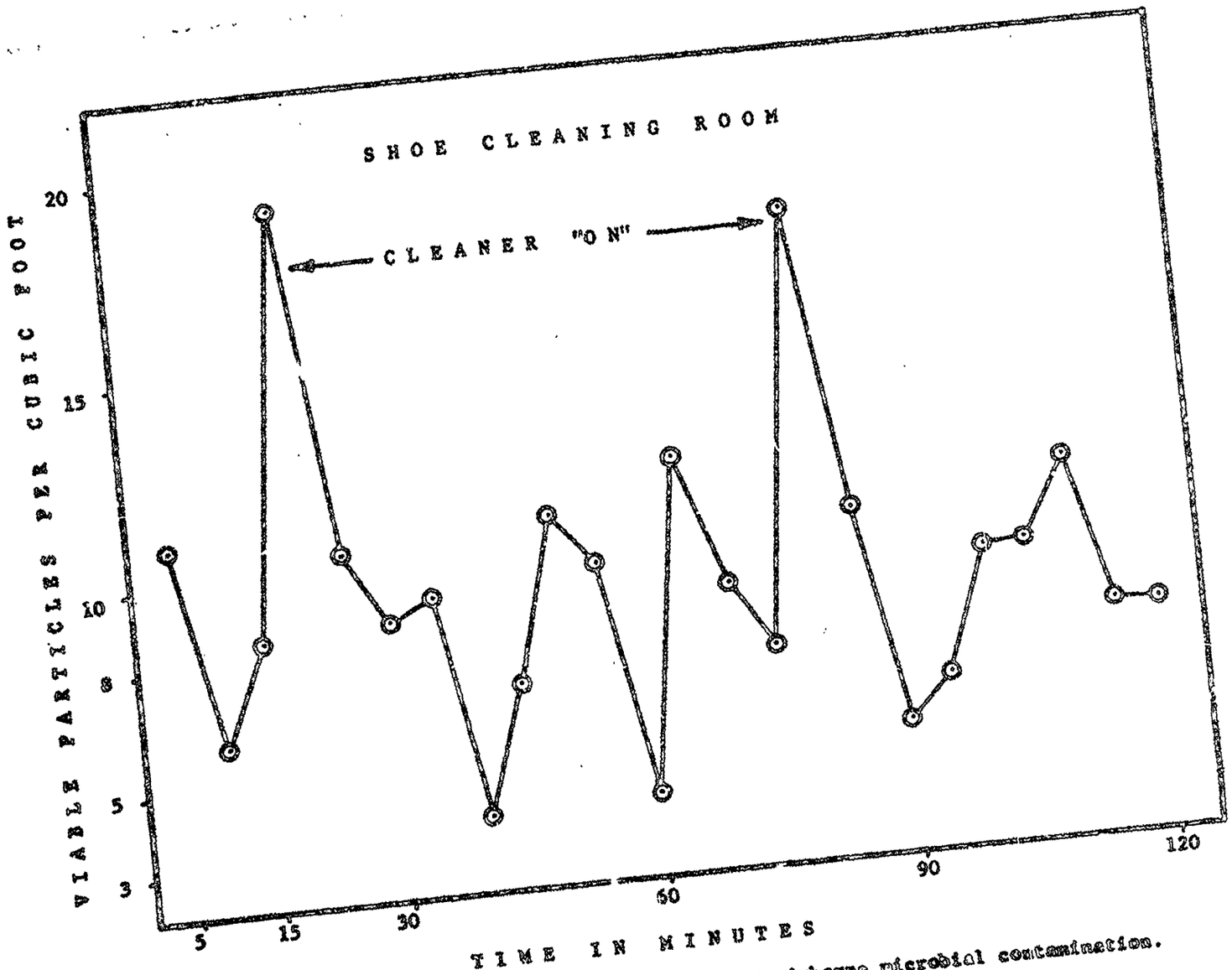


Fig. 7. Effect of shoe cleaning operation on the level of airborne microbial contamination.

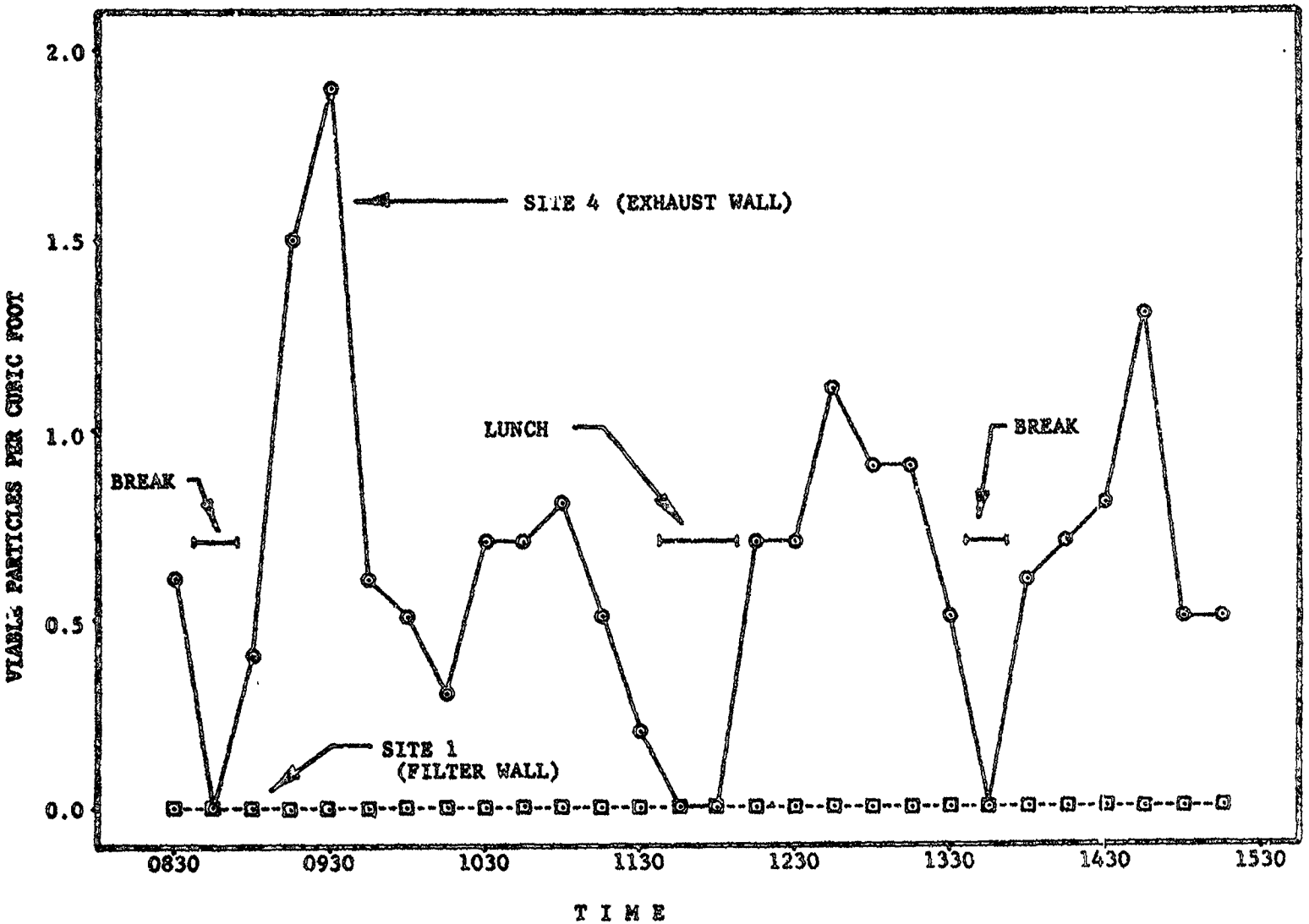


Fig. 8. Airborne microbial contamination within Clean Room D.

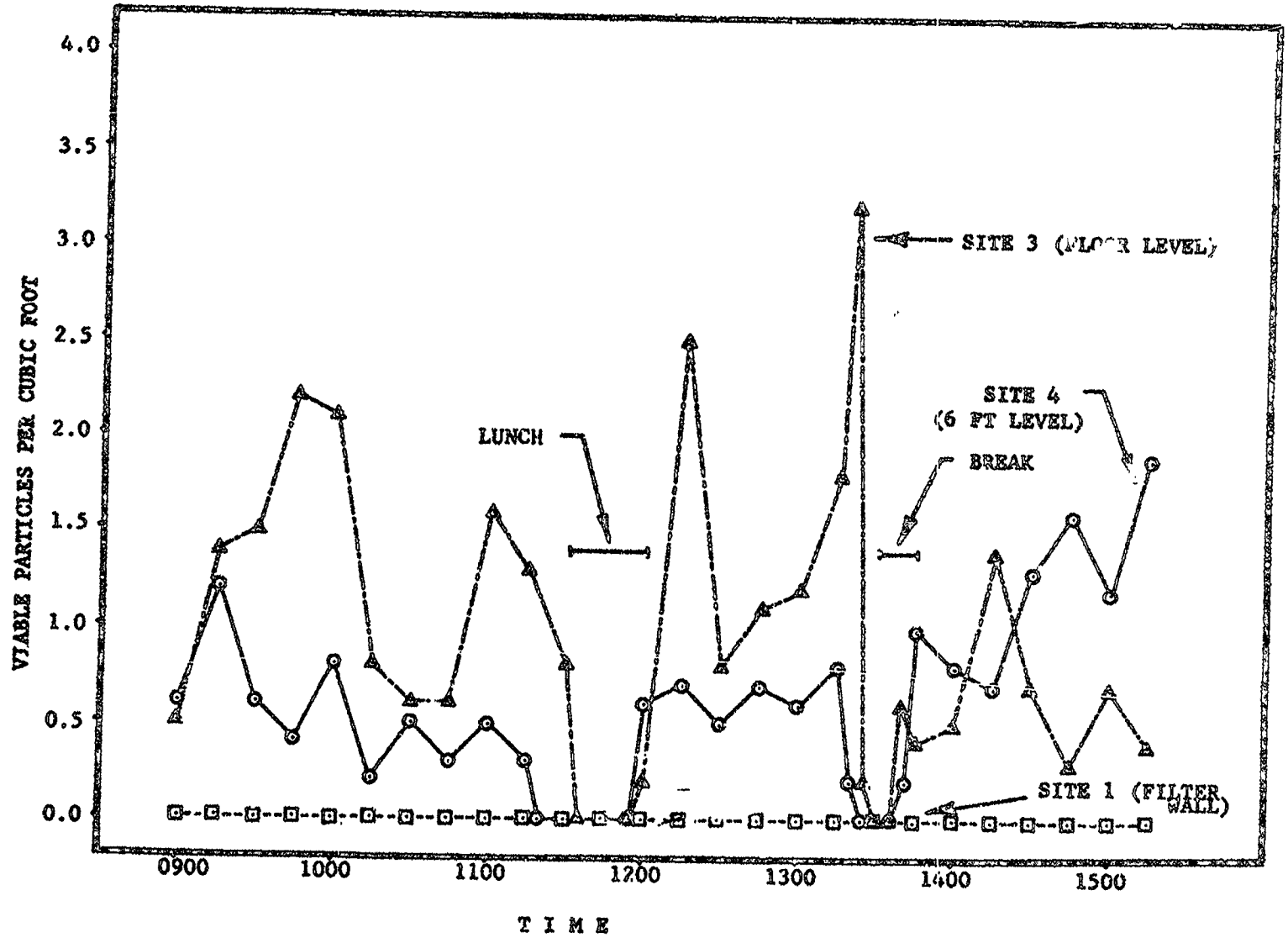
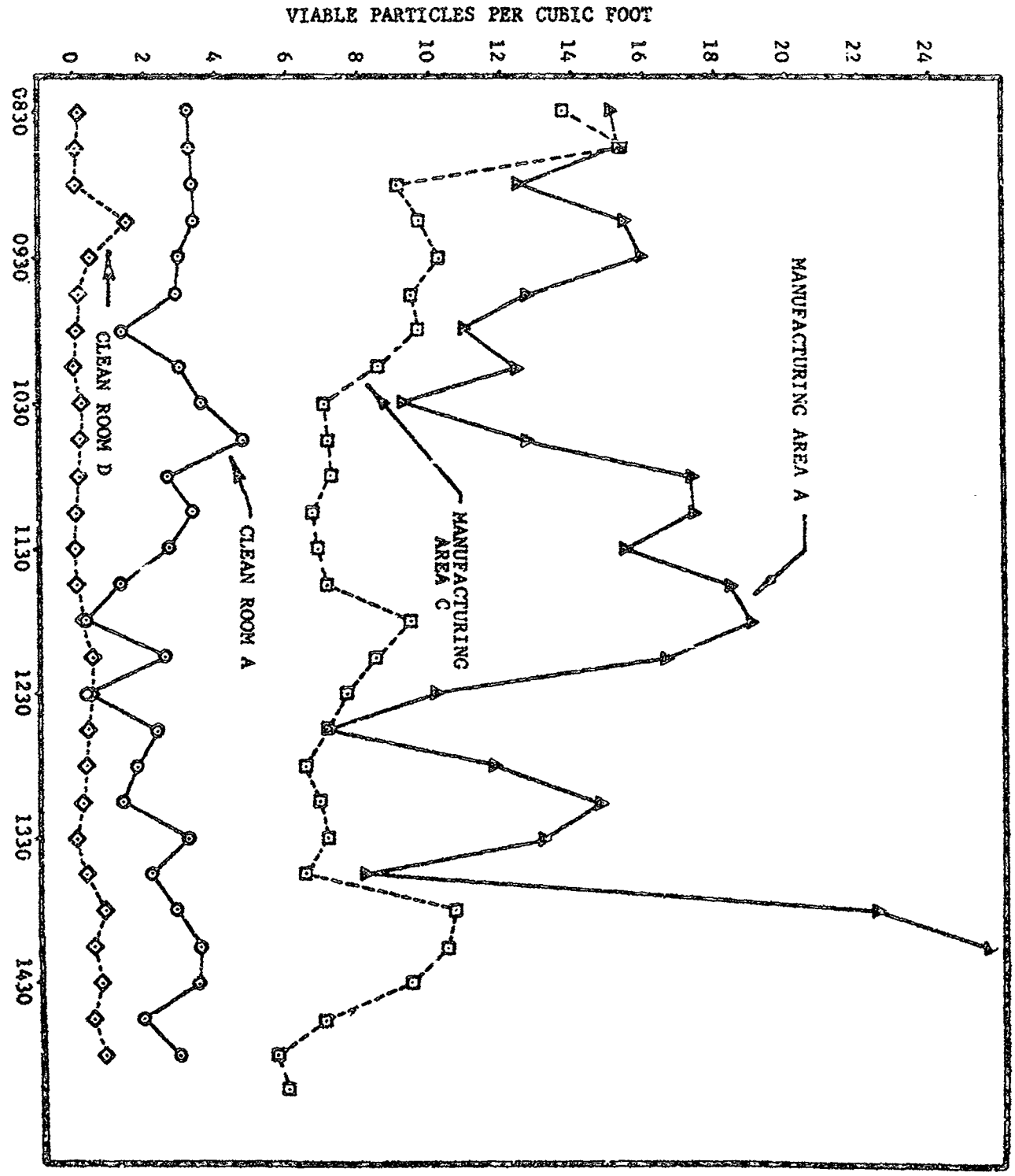


Fig. 9. Levels of airborne microbial contamination at different heights and sites in Clean Room D.

Fig. 10. Comparative levels of airborne microbial contamination in two manufacturing areas, a conventional clean room, and the exhaust wall of a horizontal laminar flow clean room.



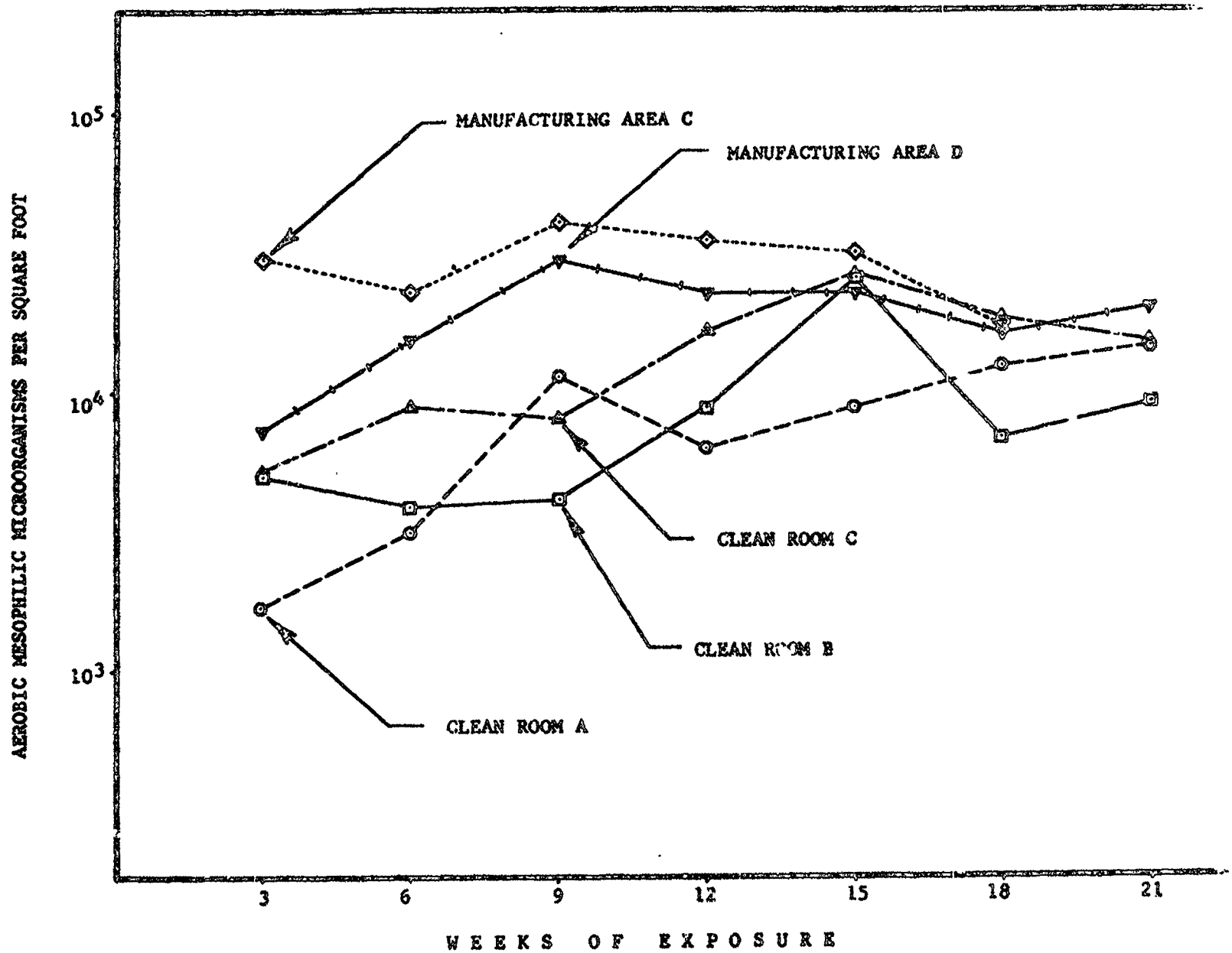


Fig. 11. Comparative levels of airborne microbial contamination which accumulat d on stainless steel surfaces exposed in 2 manufacturing areas and 3 conventional clean rooms.

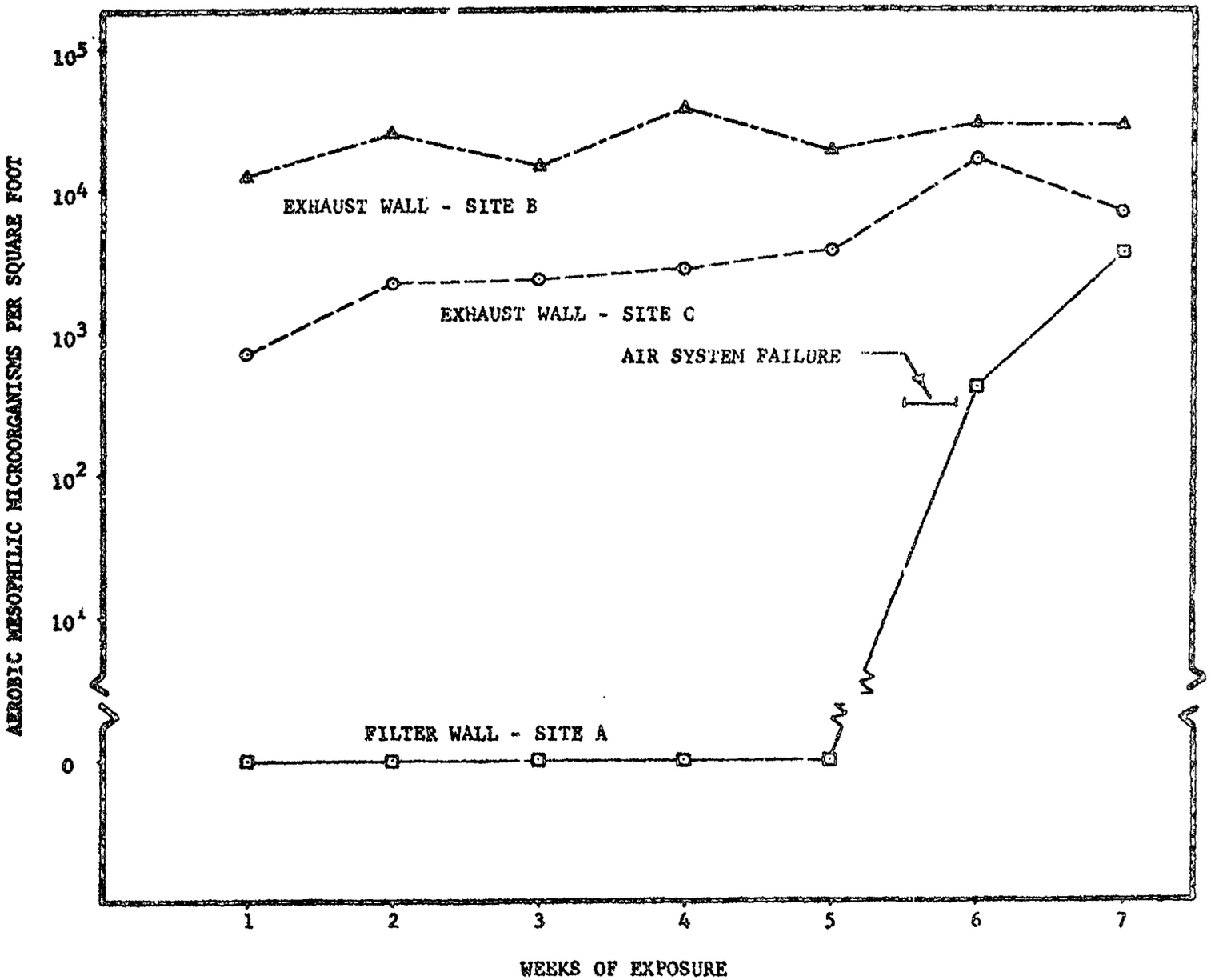


Fig. 12. Levels of airborne microbial contamination which accumulated on stainless steel surfaces exposed at 3 sites within horizontal laminar flow Clean Room D.